

The University of Chicago

A STUDY OF
THE MODE OF ATTACHMENT OF
MAGNESIUM IN CHLOROPHYLL

A DISSERTATION SUBMITTED TO THE
GRADUATE FACULTY IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF CHEMISTRY

1931

By

BRUCE JONES MILLER

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INTRODUCTION.

The study of the mode of attachment of magnesium in chlorophyll, and in derivatives of chlorophyll containing magnesium, was undertaken by me because of the discrepancy between the chemical properties of the magnesium of chlorophyll as actually observed and that to be expected from a compound in which the magnesium atom is attached to nitrogen, in accordance with the formula suggested by Willstatter.

In the investigation, a study was made of the ease of introduction of magnesium into the following magnesium free derivatives.

1. Pheophytin.
2. Ethyl pheophorbide, (a) and (b).
3. Rhodoporphyrin.
4. Pyrroporphyrin.

An investigation was also made of the ease of removal of the magnesium from the following phyllins.

1. Chlorophyll.
2. Potassium chlorophyllide.
3. Rhodophyllin.
4. Pyrrophyllin.
5. Derivative formed by reaction of magnesium acetate with pheophytin or ethyl pheophorbide.

PREVIOUS WORK.

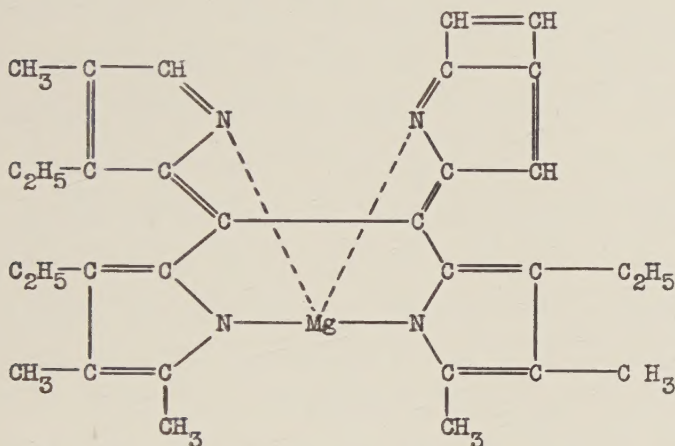
The complexly bound magnesium atom in chlorophyll, and, as a matter of fact in all the phyllins, is very sensitive to acid but extraordinarily stable toward alkali.¹

For instance, at room temperature methyl alcoholic potassium hydroxide, added to an alcoholic solution of chlorophyll, leads to the hydrolysis of the three ester groups present in chlorophyll and yields potassium chlorophyllide, a tri-potassium salt. Treatment of this salt with concentrated methyl alcoholic potassium hydroxide in a silver tube at 130° leads to the elimination of one of the carboxyl groups yielding the dicarboxylic acid, glaucophyllin. If the temperature is raised to 165°, the dicarboxylic acid, rhodophyllin, is formed. A further increase in temperature of the reacting mixture to 195 to 200° splits off a second carboxyl group yielding the monocarboxylic acid, pyrrophyllin. And finally when the pyrrophyllin is heated with soda-lime, a carboxyl-free substance, etiophyllin, is formed. During this drastic

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1. Willstatter and Hocheder, Ann., 354, 205 (1907)
Willstatter and Fritzsche, Ann., 371, 33 (1909)
Willstatter and Pfannenstiel, Ann., 358, 205 (1907)
Willstatter, Fischer, and Forsen, Ann., 400, 147 (1913)

treatment with alkali, the complexly bound magnesium atom remains intact. The structure of etiophyllin¹ as proposed by Willstatter is as follows:

ETIOPHYLLIN.



According to Willstatter the position of the two methyl groups is arbitrary, and the cyclobutane ring could be attached to the pyrrole in the position.

Whereas the phyllins are extremely stable toward alkali, all lose their magnesium under the influence of acid. The magnesium free compounds belong to the general class of chlorophyll derivatives, commonly known as the

1. Willstatter and Fischer, Ann., 400, 182 (1913)

porphyrins.

Chlorophyll and the other phyllins contain one atom of magnesium to four atoms of nitrogen. Since etiophyllin, the parent substance, contains according to the work of Willstatter, no carboxyl groups, only the nitrogen-containing groups of the molecule are available for combining with the magnesium. Willstatter rejects the idea that oxygen may play any part in the formation of the complex, and suggests that the magnesium atom is bound to two pyrrol nitrogen atoms by primary valences, and to two other pyrrol nitrogen atoms by secondary valences to form the complex. This structure is based on the theory of structure of complex metallic compounds by Werner.¹

Willstatter proposes that this magnesium complex is the same as that of other organo-magnesium compounds, differing only in degree of complexity and stability toward water. A comparison is drawn between chlorophyll and the Grignard reagent, and the fact that in the former case the magnesium atom is attached to nitrogen and in the latter case to carbon is not considered as a pronounced or characteristic difference.

1. Werner, "Neuere Anschauungen auf dem Gebiete der anorganischen Chemie", second edition, Braunschweig (1909)

The differences, however, between the linkage of the magnesium atom in the phyllins and in other organo-magnesium compounds are singular and extraordinary. As Tschelincev and Tronov¹ have pointed out, no organo-magnesium compound is known which exhibits the stability of the phyllins toward water and toward alkali. It is shown by these investigators that the analogy drawn between a Grignard reagent and the phyllins is incompatible with the inactivity of the phyllins toward water.

This apparent discrepancy between the behavior of the phyllins as actually observed, and that to be expected where the magnesium atom is bound as represented in the structure proposed by Willstatter, led Huddle² to a study of organo-magnesium compounds of various types and combinations to ascertain if any could be prepared with the same degree of stability of the magnesium atom as that exhibited by the magnesium atom in the phyllins.

The work included the testing of the stability of many compounds toward water, alkali, and acids where the magnesium atom was attached to either oxygen, nitrogen, or carbon atoms.

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1. Tschelincev and Tronov, J. Russ. Phys. Chem. Soc., 46, 1876 (1914)
 2. Huddle, Ph. D. Thesis, University of Chicago (1930)

The types of compounds investigated by Huddle^I were as follows:

Compounds containing acidic nitrogen.

1. Amides.
2. Substituted Amides.
3. Diamides.
4. Imides.

Compounds containing basic nitrogen.

5. Pyrroles.
6. Pyridines.
7. Quinolines.

Compounds of other types.

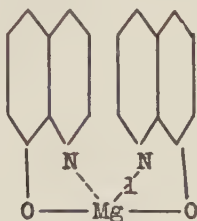
8. Hydroxy acids.
9. Magnesium alcoholates.
10. Magnesium salts of hydroxyquinolines.

In this comprehensive study of organo-magnesium compounds no substance was found, stable toward alkali, where magnesium was attached to nitrogen alone. The only magnesium complex compound approximating the stability of the phyllins was the magnesium salt of 8-hydroxyquinoline. This magnesium salt was very stable toward alkali, but unstable toward acid.

1. Huddle, Loc. cit.

Thirty hours of continued boiling with ten percent sodium hydroxide were required to completely decompose it, as compared with the phyllins which were stable in concentrated alkali at temperatures well above 200° .

The metallic linkage in the magnesium salt of 8-hydroxyquinoline is not of metal to nitrogen alone, but of metal to oxygen by a primary valence, and metal to nitrogen by a secondary valence as follows:



It is believed, however, that the stability of the salts of 8-hydroxyquinoline is not due to the presence of a complex, metal to nitrogen linkage, but rather to the insolubility of these salts in water and organic solvents.

PLAN OF PRODEEDURE.

In view of the above facts, it is believed that the relation of the magnesium atom to the chlorophyll molecule is still an open question. The observed behavior of chlorophyll does not agree with the structure as proposed by Willstatter. It was because of this apparent discrepancy that the study of the mode of attachment of the magnesium atom in chlorophyll and its derivatives was undertaken. The investigation began with a study of the ease of introduction of magnesium into the following porphyrins:

1. Pheophytin.
2. Ethyl pheophorbide (a) and (b).
3. Rhodoporphyrin.
4. Pyrroporphyrin.

The ease of removal of magnesium from the following phyllins was also studied:

1. Chlorophyll.
2. Potassium chlorophyllide.
3. Rhodophyllin.
4. Pyrrophyllin.
5. Derivative formed by the reaction of magnesium

acetate with pheophytin or ethyl pheophorbide.

Introduction of Magnesium into Pheophytin.

I

Pheophytin, the magnesium free derivative of chlorophyll

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1. Willstatter, "Investigations on Chlorophyll", translated by Schertz and Merz, 230 (1928).

was prepared by treating an alcoholic solution of crude chlorophyll with hydrochloric acid. The pheophytin precipitates from the alcoholic solution in a pure state, and is ash-free.

I
Willstatter succeeded in preparing pure unallomerized chlorophyll from this magnesium free derivative by the introduction of magnesium into the molecule with methyl magnesium iodide. The synthetic product gave the brown phase characteristic of pure undamaged chlorophyll. And analysis showed no difference in the magnesium or phytol content or in the COOCH_3 group for they were present in the same ratios as in chlorophyll itself. The absorption spectra, however, was not taken. Willstatter² also found that he could introduce magnesium into pheophytin with magnesium oxide in the presence of concentrated methyl alcoholic potash. High temperatures had to be employed and consequently the formation of chlorophyll itself was excluded. Various phyllins were formed depending on the temperatures employed. The method successful for the introduction of the heavy metals into pheophytin was much more

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1. Willstatter and Forsen, Ann., 396, 180 (1913).
 2. Willstatter and Forsen, Ann., 396, 180 (1913).

simple.¹ Treatment of an alcoholic solution of pheophytin with lead acetate or cupric acetate introduced the metal in complex combination immediately. It is merely stated that magnesium could not be introduced by this method, because of the greater instability of the magnesium complex to acid.

Since salts of weak acids produce a very slight increase in hydrogen ion concentration in alcoholic solution, attempts were made to reintroduce magnesium into pheophytin using the magnesium salt of a weak acid in alcoholic solution. Our experience, when magnesium salts of strong acids were used, such as magnesium chloride and magnesium sulfate, was that even at high temperatures the magnesium complex did not form, for the pheophytin was recovered unchanged. Likewise, when pheophytin was treated with colloidal magnesium hydroxide at elevated temperatures, no change took place, due probably to the very slight solubility of colloidal magnesium hydroxide in alcohol. However, when pheophytin was treated with magnesium acetate in alcoholic solution at 150° for five hours, a very definite change was observed. The solution had changed from the brown color characteristic of pheophytin to a deep green color with a beautiful red fluorescence. The reaction product was recovered and purified by transference to ether, and then washing the ether solution with distilled

1. Willstatter and Sjöberg, *Z. Physiol. Chem.*, 138, 171 (1924)

water to remove the magnesium acetate and alcohol. The product purified by this method, gave by analysis only two to three percent of ash. But, if the residue, left upon evaporation of the purified ether solution, was dissolved in a small amount of ether and precipitated with low boiling point ligroin, the ash content was increased. This process of purification removed some ash free impurities including phytol, which was formed by partial replacement with an ethoxy grouping under reaction conditions. That free phytol was formed, was demonstrated by the fact that in drying the residue at elevated temperatures in vacuo, a yellow, viscous liquid condensed on the cooler portions of the drying apparatus. By purification with low boiling point ligroin, the ash content of this unknown chlorophyll derivative was increased to four percent. The variation in magnesium content of the phyllin prepared by this method was not greater than four tenth of one percent.

Introduction of Magnesium into Ethyl Pheophorbide.

Ash-free ethyl pheophorbide¹ was prepared by the acid hydrolysis of pure pheophytin in ethyl alcohol. The (a) and (b) ethyl chlorophyllides were partially separated by fractional precipitation from ether.

1. Willstatter, "Investigations on Chlorophyll", transl. by Schertz & Merz, 254 (1928).

An alcoholic solution of ethyl chlorophyllide (a) or ethyl chlorophyllide (b) when treated with magnesium acetate at 150° yielded a magnesium containing chlorophyll derivative, which analyzed approximately the same for ash, the ash consisting of pure magnesium oxide. It is interesting to compare the percentage of ash obtained in the synthetic products with that of ethyl chlorophyllide prepared from chlorophyll. In the former the percentage of ash varied between 4.8 and 5.7 percent, while in a pure sample of the latter the percentage of ash is 6.2.

An Attempt to Identify the Unknown Chlorophyll
Derivative by Absorption Spectra.

An attempt was made to identify the synthetic phyllin by a comparison of its absorption spectra with that of known compounds. The absorptionspectra of an (a) and (b) mixture of ethyl pheophorbide which had been treated with magnesium acetate in alcoholic solution,¹ and purified in the usual manner, was compared with that of the following:

- (1) chlorophyll (a) and (b) mixture,
- (2) chlorophyll heated in ethyl alcohol,
- (3) chlorophyll heated in ethyl alcohol with
magnesium acetate,

1. On plate I this preparation is called synthetic chlorophyll.

(4) the magnesium free derivative, ethyl pheophorbide.

The absorption spectra of these compounds were taken on a Steinheil Spectrograph. These absorption spectra are found on plate III. On plate I the inverse of the intensity of absorption is plotted against wave length. The intensity of absorption was obtained by means of a recording microphotometer. A careful comparison of the absorption spectra of chlorophyll with the unknown synthetic chlorophyll derivative shows very slight differences in the two phyllins. The differences are accentuated in the curves on plate I. While Willstatter merely compares absorption bands for identification, here the relative intensity of absorption is compared throughout the region of exposure. This method, therefore, of plotting the inverse of the intensity against wave length is a far better test of the identity of two compounds than the method employed by Willstatter.

It can be seen at a glance that there is no close agreement between the spectra of any two preparations. Even chlorophyll and chlorophyll heated in ethyl alcohol show marked differences, due no doubt to allomerization of the chlorophyll molecule which was treated with ethyl alcohol. This process of allomerization is assumed by Conant¹ to

1. Conant, Dietz, Bailey, and Kamerling, J. Am. Chem. Soc., 53, 2382 (1931)

be a process of dehydrogenation by atmospheric oxygen in which an α -hydroxy acid grouping is transformed to an α -ketonic acid grouping.

These results would indicate that, undoubtedly, slight differences in structure produce very great changes in absorption spectra, and consequently this method did not lend itself to the identification of the unknown synthetic phyllin.

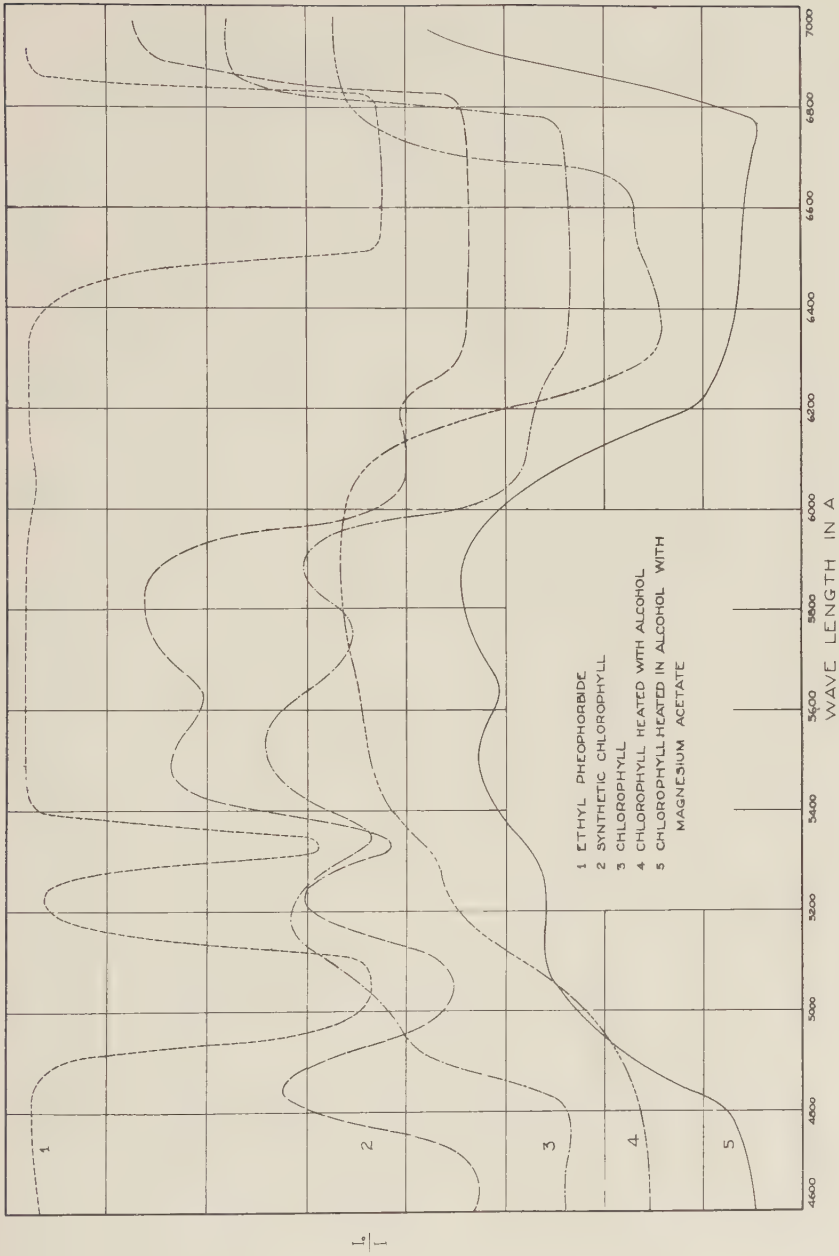


PLATE I

The Partial Synthesis of Glaucophyllin.

That the magnesium atom in the unknown synthetic phyllin was complexly bound as in the naturally occurring phyllins was finally proved by the decomposition of the unknown compound with concentrated methyl alcoholic potassium hydroxide. By this procedure glaucophyllin, a dicarboxylic acid, was formed and isolated. The analysis for magnesium agreed with that for glaucophyllin prepared from chlorophyll, and its absorption spectra agreed with that of a known sample of glaucophyllin. The absorption spectra data is recorded on plates II and III. In plate two the general shape of the curves agree perfectly. The data for these curves was obtained in the same way as that for plate I.

The foregoing data shows that the magnesium free chlorophyll derivatives, pheophytin and ethyl pheophorbide, can be treated with magnesium acetate in alcoholic solution to reintroduce the magnesium atom in the same position it occupies in the naturally occurring chlorophyll. Of the magnesium salts tried, magnesium acetate was the only salt that permitted this. Magnesium chloride and magnesium sulfate gave no reaction. The singular effect of the magnesium acetate is probably due to the fact that it is the salt of a weak acid, the hydrolysis of which salt



PLATE II

ABSORPTION SPECTRA.

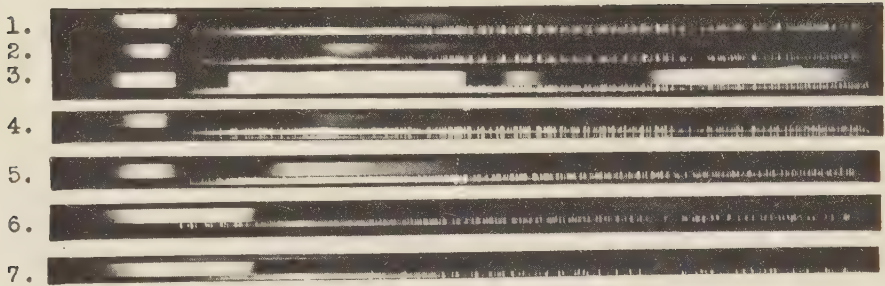


Plate III.

1. Chlorophyll.
2. Unknown chlorophyll derivative.
3. Ethyl pheophorbide.
4. Chlorophyll heated in alcohol with magnesium acetate.
5. Chlorophyll heated with alcohol.
6. Synthetic glaucophyllin.
7. Glaucophyllin.

produces only a very slight increase in hydrogen ions to which the phyllins are unusually sensitive. It is reasonable to assume that the magnesium salt of any other weak acid, soluble in alcohol, would produce the same results.

It is believed that the method here outlined for the introduction of magnesium is general and can be used with all porphyrins. It has been studied, however, only in two other cases.

Introduction of Magnesium into Rhodoporphyrin.

A sample of rhodoporphyrin was dissolved in pyridine, and ethyl alcohol and magnesium acetate were added. The solution was heated at 150° for five hours. After purification of the product formed, it was analyzed for ash. This analysis showed that the magnesium content of the compound formed was close to that found in rhodophyllin prepared from chlorophyll. No further tests were made to identify the product.

Introduction of Magnesium into Pyrroporphyrin.

A suspension of pyrroporphyrin and magnesium acetate in absolute alcohol was heated as above. The reaction mixture was purified in the usual manner and the percentage of ash was low as compared with that of pyrrophyllin prepared from chlorophyll. No further tests were made to identify the product.

Stability of Magnesium in Chlorophyll and its
Derivatives.

It has been shown by Willstatter¹ that under the influence of acid the phyllins lose their magnesium and yield polybasic or monobasic carboxylic acids. But in the presence of alkali, even at very high temperatures the magnesium retains its position in the complex form in the molecule.

During the course of this investigation attempts were made to remove the magnesium atom from chlorophyll, and a few of its derivatives in alkaline solution as the magnesium salt of 8-hydroxyquinoline.

An alcoholic solution of chlorophyll, made alkaline with ammonium hydroxide, was treated with 8-hydroxyquinoline. No precipitation of the magnesium salt of 8-hydroxyquinoline took place even after the solution had stood at room temperature for several weeks. A similar attempt was made to remove the magnesium from the unknown phyllin, prepared by treating either pheophytin or ethyl pheophorbide with magnesium acetate in alcohol. Here no magnesium was removed after one month. Rhodophyllin and pyrrophyllin with similar treatment gave negative results. However, when potassium chlorophyllide was subjected to the above treatment, the

1. Willstatter and Hocheder, Ann., 354, 205 (1907)

magnesium was removed from the phyllin quantitatively as the magnesium salt of 8-hydroxyquinoline.

It is most remarkable that potassium chlorophyllide is the only derivative of chlorophyll that gave up its magnesium to 8-hydroxyquinoline. The fact that pyrrophyllin, a monocarboxylic acid, and rhodophyllin, a dicarboxylic acid, retain their magnesium when treated with 8-hydroxyquinoline, whereas chlorophyllide, a tricarboxylic acid, loses its magnesium quantitatively is suggestive of a more profound change in structure in going from chlorophyll to potassium chlorophyllide than has been suggested by any investigators of chlorophyll. The results are also suggestive of a profound change in structure in going from chlorophyllide, a tricarboxylic acid, to rhodophyllin, a dicarboxylic acid. The structures suggested for the magnesium complex in the phyllins does not disclose even a remote clew to the solution of this fact.

EXPERIMENTAL PART

An Attempt to Introduce Magnesium into Pheophytin with Colloidal Magnesium Hydroxide.

To 0.11 grams of pheophytin, 0.20 grams of colloidal magnesium hydroxide¹ and 40 cc. of 95% ethyl alcohol were added. No reaction had taken place after the mixture had been agitated two days at room temperature; nor was there any reaction after the suspension had been heated five hours at either 100 or 150°, for the pheophytin was recovered unchanged. After the above treatment the mixture was filtered into approximately 250 cc. of ether. The ether solution was washed thoroughly eight times with distilled water to remove the alcohol and traces of colloidal magnesium hydroxide. During each washing a small quantity of black amorphous material separated from the ether layer. This material was discarded. The ether solution was then filtered and evaporated to dryness. The residue was transferred to a weighed crucible and dried twelve hours in vacuo over sulfuric acid. The residue, when ignited, left no detectable quantity of ash.

An Attempt to Introduce Magnesium into Pheophytin in

1. Prepared by treating magnesium oxide with water at 150 . Mellor, "A Comprehensive Treatise on Inorganic and Theoretical Chemistry", Vol. 4, 290 (1923).

Chloroform Solution with Magnesium Acetate.

To a solution of 0.10 grams of pheophytin in 50 cc. of chloroform, 0.05 grams of magnesium acetate and 0.20 grams of colloidal magnesium hydroxide were added. The mixture was heated five hours at 100° , and then five hours at 150° . There was no reaction and the pheophytin was recovered unchanged. The mixture was filtered and washed thoroughly seven times with distilled water to remove the magnesium acetate and traces of colloidal magnesium hydroxide. The chloroform solution was filtered and evaporated to dryness. The residue was transferred to a crucible of known weight and dried for a day in vacuo over sulfuric acid and sodium hydroxide. The residue, which weighed 0.0892 grams, burned completely leaving no ash.

An Attempt to Introduce Magnesium into Ethyl Pheophorbide¹ with Magnesium Chloride.

To a 0.30 gram sample of ethyl pheophorbide, 0.40 grams of magnesium chloride, and 150 cc. of 95% ethyl alcohol were added. The mixture was sealed in a silver bomb tube and heated five hours at 150° . Half of the solution

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1. Ethyl and methyl pheophorbide were prepared by the acid hydrolysis of pheophytin, according to the method of Wilstatter. Wilstatter, "Investigations on Chlorophyll", transl. by Schertz & Merz, 254 (1928).

was then removed and filtered into 300 cc. of ether. The ether solution was then washed thoroughly with distilled water until all of the alcohol and magnesium chloride were removed. The ether solution was filtered and evaporated to dryness. The residue was transferred to a weighed crucible and dried for a day in vacuo over sulfuric acid. The residue weighed 0.1580 grams and burned completely leaving no ash. The other half of the alcoholic solution was resealed in the silver bomb tube and heated 24 hours at 145 to 150°. The pheophytin was separated from the magnesium chloride and alcohol as described directly above. The residue was dried for a day in vacuo over sulfuric acid. It weighed 0.1232 grams, and when ignited left no ash.

An Attempt to Introduce Magnesium into Ethyl Pheophorbide with Magnesium Sulfate.

To 150 cc. of alcohol were added 0.40 grams of magnesium sulfate and 0.30 grams of ethyl pheophorbide. The mixture was placed in a silver bomb tube and heated 24 hours at 145 to 150°. The contents of the tube were then filtered into ether and purified in the usual manner. During the washing of the ether solution with water considerable quantities of black amorphous material separated from the ether layer and were discarded. After filtering the ether solution and evaporating to dryness, the residue that remained

was transferred to a weighed crucible and dried 24 hours in vacuo over sulfuric acid. The residue which weighed 0.2096 grams gave no ash when burned.

Introduction of Magnesium into Pheophytin in Alcohol with Magnesium Acetate.

To a suspension of 0.15 grams of pheophytin in 75 cc. of ethyl alcohol, 0.10 grams of magnesium acetate and 0.05 grams of colloidal magnesium hydroxide were added. The mixture was sealed in a pyrex bomb tube and heated five hours at 145 to 150°. At the end of that time, the characteristic brown colored solution had changed to a deep green color with a beautiful red fluorescence. The contents of the tube were filtered into ether. The ether solution was washed thoroughly many times to remove the alcohol, magnesium acetate, and traces of colloidal magnesium hydroxide. During the washing small quantities of black amorphous material separated from the ether layer and were discarded. The ether solution was filtered and evaporated to dryness. The residue, bluish-black in color, was transferred to a weighed crucible, and dried to constant weight in vacuo over sulfuric acid. The residue was ignited and left a white fluffy ash that gave all the tests for magnesium.

Anal. Subs., 0.1238: Ash, 0.0028. Calculated for chlorophyll: Ash, 4.5. Found 2.2.

Four tests carried out as described directly above gave an average percentage of ash of 2.2. These analysis varied six-tenth of one percent.

The colloidal magnesium hydroxide took no part in the reaction since the same results were obtained when colloidal magnesium hydroxide was not used along with the magnesium acetate.

To 0.15 grams of pheophytin suspended in 75 cc. of 95% ethyl alcohol, 0.15 grams of magnesium acetate was added. The mixture was treated as described directly above for the preparation in which colloidal magnesium was used in conjunction with magnesium acetate. The ether soluble residue obtained was dried to constant weight in vacuo over sulfuric acid. It was then ignited and the percentage of ash determined.

Anal. Subs., 0.1372: Ash, 0.0036. Calcd. for chlorophyll: Ash, 4.5. Found 2.6.

In succeeding experiments, carried out as described above, the residue was dried over a boiling toluene bath at a pressure of two millimeters of mercury. By this method of drying, the residue, which in one case originally weighed 0.1858 grams after drying over sulfuric acid, lost two to four milligrams in weight per day for several weeks. The final weight of the residue was 0.1480 grams. This residue

was burned and left a white ash.

Anal. Subs., 0.1480: Ash, 0.0048. Calcd. for chlorophyll: Ash, 4.5. Found 3.2.

The ash from a sample of ignited chlorophyll should consist of pure magnesium oxide. It was thought advisable to test the purity of the ash in these synthetic preparations. This was done by dissolving the ash in dilute hydrochloric acid with consequent precipitation of the magnesium with 8-hydroxyquinoline, according to the method of Berg.¹

An analysis by this method was made of the above sample of ash.

Anal. Subs., 0.0048: $\text{Mg}(\text{C}_9\text{H}_6\text{ON})_2$, 0.0358. Calcd. for $\text{MgO}:\text{Mg}$, 0.0029. Found 0.0028.

The analysis shows that the ash consisted of pure magnesium oxide.

The ash or magnesium oxide content of the above preparations was low. It was found that the magnesium containing compound could be partially separated from accompanying magnesium-free impurities by the precipitation of the synthetic chlorophyll derivative from its concentrated ether solution by the addition of a large volume of low boiling point ligroin. This process of purification, if repeated several times, increased the magnesium content of the portion insoluble in ligroin to four percent. The analysis of such a preparation

1. Berg, Z. Anal. Chem., 70, 341 (1927)

follows.

Anal. Subs., 0.1698: Ash, 0.0066. Calcd. for chlorophyll: Ash, 4.5. Found 4.0.

Introduction of Magnesium into Ethyl Pheophorbide with Magnesium Acetate.

To 0.20 grams of ethyl pheophorbide (a),¹ 0.40 grams of magnesium acetate and 100 cc. of 95% ethyl alcohol were added. This mixture was sealed in a pyrex bomb tube and heated five hours at 145 to 150°. At the end of this time the brown colored solution characteristic of ethyl pheophorbide (a) had changed to a deep green color with a red fluorescence. The contents of the tube were filtered into ether, and washed thoroughly ten times with distilled water to remove the alcohol and magnesium acetate. As soon as the ether solution became poor in alcohol, a small quantity of black amorphous solid material separated from the ether layer with each succeeding washing with distilled water. This material was discarded. The ether solution was filtered, after it was certain that the solution was free of magnesium acetate and alcohol, and evaporated to dryness. The residue was transferred to a weighed crucible, and dried to constant weight in vacuo at 100°. The ignited residue left a white

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1. Ethyl pheophorbide (a) used in this and succeeding experiments was contaminated with small amounts of the corresponding (b) compound. Willstatter, "Investigations on Chlorophyll" transl. by Schertz & Merz, 254, (1928).

ash.

Anal. Subs., 0.1340: Ash, 0.0034. Calcd. for ethyl pheophorbide. Ash, 6.2. Found 2.4.

The technique was changed and the percentage of magnesium was increased.

To 0.30 grams of ethyl pheophorbide (a), 0.60 grams of magnesium acetate was added. This mixture was suspended in 150 cc. of ethyl alcohol in a silver bomb tube. The tube was heated five hours at 145 to 150°. The same change in the color of the solution took place as above. The substance formed was filtered into ether and purified in the usual manner. The ether soluble residue was dissolved in a few cc. of ether, and 200 cc. of low boiling point ligroin was added. This precipitated the supposedly, ethyl chlorophyllide in an easily filterable form. The precipitate was collected, dissolved again in a small quantity of ether, and again precipitated with low boiling point ligroin. This process was repeated a third time. The precipitate was transferred to a weighed crucible and dried in vacuo at 100°. The ignited residue left a white ash.

Anal. Subs., 0.0870: Ash, 0.0042. Calcd. for ethyl chlorophyllide: Ash 6.2. Found 4.8.

Introduction of Magnesium into Ethyl Pheophorbide (b)
with Magnesium Acetate.

A 0.20 gram sample of ethyl pheophorbide (b),¹ and 0.40 grams of magnesium acetate were added to 100 cc. of 95% ethyl alcohol. The mixture was heated in a pyrex bomb tube five hours at 145 to 150°. The contents of the tube were then filtered into ether and purified in the usual way. The ether soluble residue was dried to constant weight in vacuo at 100°. The residue was burned and the percentage of ash determined.

Anal. Subs., 0.1218: Ash, 0.0061. Calcd. for ethyl chlorophyllide: Ash, 6.2. Found 5.0.

In another preparation the product was purified by the use of low boiling point ligroin.

A 0.030 gram sample of ethyl pheophorbide (b) and 0.60 grams of magnesium acetate were added to 150 cc. of ethyl alcohol. The mixture was heated five hours at 145 to 150° in a silver bomb tube. The usual change in color of the solution took place. The contents of the tube were filtered into ether, and purified in the usual manner. The ether solution was filtered and evaporated to dryness. The residue was taken up in the least possible amount of ether. Low boiling point ligroin was then added slowly to the ether solution until 200 cc. had been added. The magnesium containing compound precipitated, and was filtered off. This

1. Ethyl pheophorbide (b) used in this and succeeding experiments was contaminated with a small amount of the corresponding (a) compound.

precipitate was transferred to a weighed crucible and dried for two days in vacuo over phosphorus pentoxide. At the end of this time constant weight had been obtained. The residue was then ignited over a blast lamp and the percentage of ash determined.

Anal. Subs., 0.1211: Ash, 0.0068. Calcd. for ethyl chlorophyllide: Ash, 6.2. Found 5.6.

Attempted Identification of Chlorophyll Derivatives
by Absorption Spectra.

The following compounds were prepared for a comparison of their absorption spectra. It was hoped that the unknown compound prepared above might be identified by this method.

1. Chlorophyll.

This preparation was made according to the method of Willstatter¹ and Stoll. The product obtained is a pure chlorophyll (a) and (b) mixture. A solution of 0.0430 grams of this preparation in a liter of ether was used for the absorption spectra data. The pyrex tube in which it was placed was 100 mm. long.

2. Chlorophyll heated in ethyl alcohol at 150 for five hours.

A 0.20 gram sample of chlorophyll prepared as in

1. Willstatter and Stoll, "Investigations on Chlorophyll", transl. by Scherz & Merz, 120 (1928).

(1) above, was sealed with 100 cc. of 95% ethyl alcohol in a pyrex bomb tube and heated five hours at 145 to 150 . The alcoholic solution was filtered into ether, and washed thoroughly many times with distilled water to remove the alcohol. The ether solution was then filtered and evaporated to dryness. The residue was taken up in the least possible amount of ether, and the chlorophyll precipitated from it with low boiling point ligroin. The precipitate was dried and used for absorption spectra data. The concentration of the solution used was 0.0430 grams per liter of ether. The pyrex tube in which it was placed was 100 mm. long.

3. Chlorophyll heated in ethyl alcohol with magnesium acetate for five hours at 145 to 150⁰.

To a 0.20 gram sample of chlorophyll, prepared as in (1) above, 0.40 grams of magnesium acetate, and 100 cc. of 95% ethyl alcohol were added. The mixture was placed in a pyrex bomb tube and heated five hours at 145 to 150⁰. The contents of the tube were then removed and purified as in (2) above. A solution of 0.0430 grams per liter of ether was made up for the absorption spectra. The pyrex tube in which it was placed was 100 mm. long.

4. Ethyl pheophorbide.

This preparation was made by the method described by Schertz and Merz.¹ Pheophytin was treated with alcoholic

1. Willstatter, "Investigations on Chlorophyll", transl. by Schertz & Merz, 254, (1928).

hydrochloric acid which caused a replacement of a phytol group with an ethyl group, yielding ethyl pheophorbide. The alcoholic hydrochloric acid solution was transferred to ether, and the ether solution was washed free of alcohol and hydrochloric acid with much water. The ethyl pheophorbides (a) and (b) precipitated together when the solution was greatly concentrated. This (a) and (b) mixture was used for absorption spectra data. The concentration of the solution used was 0.0300 grams dissolved in one liter of ether. The length of the pyrex tube through which the absorption spectra was taken was 100 mm.

5. Synthetic chlorophyll. (Supposedly ethyl chlorophyllide)

The synthetic chlorophyll, so-called merely because it contained complexly bound magnesium, was prepared from a portion of the ethyl pheophorbide prepared in (4) above. This preparation was carried out in the usual manner. The purification process was the same as in (1) above. For absorption spectra data, a solution of 0.0300 grams per liter of ether was made up. This solution was placed in a 100 mm. tube for the exposure on the spectrograph.

The above unknown chlorophyll derivative was analyzed for ash.

Anal. Subs., 0.1948: Ash, 0.0110. Calcd. for ethyl chlorophyllide: Ash, 6.2. Found 5.6.

The absorption spectra of these compounds are found on plate I. The spectrograph was a Steinheil, München. The source of light was a 500 watt tungsten filament, projection lamp. The lamp was placed in a metal container which had a slit one-fourth inch wide and several inches long as the light source. For the location of the absorption bands of these preparations, an iron arc exposure was taken adjacent to the absorption spectra of each compound as shown in plate III. Special rapid Ilford pancromatic plates were used. The exposures for the preparations on plate I, were 70 seconds in each case, and were taken through a solution in a pyrex tube 100 mm. long. The absorption spectra of each preparation was run through a recording microphotometer, enabling us thus to plot the length of the plate against I/I . By extrapolation of a curve in which the length of the plate was plotted against wave length, the value of I/I could be plotted directly against wave length. This was done in the curves found on plates I and II.

The Partial Synthesis of Glaucophyllin.

By the introduction of magnesium into ethyl pheophorbide with magnesium acetate, 0.80 grams of a chlorophyll derivative was prepared. The method of preparation and purification has been previously described.

This derivative was placed in 35 cc. of 35% methyl

alcoholic potassium hydroxide, and sealed in a silver bomb tube. The tube was allowed to stand over night at room temperature. The following morning the tube was heated at a temperature of 138 to 142° for 6 $\frac{1}{2}$ hours. The contents of the tube were then removed and treated as described by Willstatter and Fritzsche.¹ A compound was isolated which was precipitated twice from anhydrous ether. Elongated rhombic crystals were obtained that resembled glaucophyllin. The yield was 0.51 grams. The compound was dried to constant weight at 105 and a pressure of two millimeters of mercury.

The glaucophyllin was analyzed for ash and for magnesium.

Anal. Subs., 0.0430: Ash, 0.0030. Calcd. for glaucophyllin: Ash as MgO, 7.1. Found 7.0.

The composition of the ash as magnesium oxide was confirmed by analysis of the ash with 8-hydroxyquinoline. The ash was brought into solution with a few drops of dilute hydrochloric acid and then precipitated as the magnesium salt of 8-hydroxyquinoline, according to the method of Berg.²

Anal. Subs., 0.0030: $\text{Mg}(\text{C}_9\text{H}_6\text{ON})_2$, 0.0228; Calcd. for MgO: Mg, 60.3. Found 59.3.

A solution of 0.0120 grams of glaucophyllin in 500 cc.

1. Willstatter and Fritzsche, Ann. 371, 62 (1909)

2. Berg, Loc. cit.

of ethyl alcohol was prepared. The absorption spectra of this solution was taken in a 100 mm. pyrex tube, and compared with that of the known compound prepared according to the method of Willstatter and Fritzsche.¹ The results are found on plate II. The absorption spectra of the known and unknown compound are in close agreement. The absorption spectra were taken under exactly the same conditions as for those substances recorded on plate I, except that in this case the length of exposure was 30 seconds.

Introduction of Magnesium into Pyrroporphyrin with Magnesium Acetate.

To 50 cc. of absolute alcohol, 0.071 grams of pyrroporphyrin and 0.15 grams of magnesium acetate were added. The suspension was heated in a pyrex bomb tube for five hours at 145 to 150°. The contents of the tube were filtered into 250 cc. of ether. The ether solution was washed five times with dilute mono-sodium phosphate to decompose any magnesium salt of pyrroporphyrin that might have formed during the heating. The solution was then washed thoroughly many times with distilled water to remove the alcohol and the mono-sodium phosphate. The ether solution was evaporated to dryness, and the residue transferred to a

1. Willstatter and Fritzsche, Ann., 371, 62 (1909)

weighed crucible. The residue was dried to constant weight in vacuo over phosphorus pentoxide, and then analyzed for ash.

Anal. Subs., 0.0372: Ash, 0.0024. Calcd. for pyrrophyllin: Ash, 6.5. Found 6.4.

The purity of the ash, as magnesium oxide, was determined by dissolving the ash in dilute hydrochloric acid, and precipitating the magnesium as the salt of 8-hydroxyquinoline according to the method of Berg.¹

Anal. Subs., 0.0024: $\text{Mg}(\text{C}_9\text{H}_6\text{ON})_2$, 0.0122. Calcd. for MgO : Mg, 0.00145. Found 0.0008.

The pyrrophyllin crystallizes with eleven percent of ether of crystallization. The percentage of ash in pyrrophyllin was calculated on this basis.

Introduction of Magnesium into Rhodoporphyrin with Magnesium Acetate.

To 50 cc. of absolute ethyl alcohol, 0.070 grams of rhodoporphyrin and 0.15 grams of magnesium acetate were added. The mixture was placed in a silver bomb tube and heated five hours at 145 to 150°. The contents of the tube were then removed and examined. The absolute alcohol was

1. Berg, Z. Anal. Chem., 70, 341 (1927)

only slightly red in color. There was a large amount of reddish-black, alcohol insoluble residue. This was partly soluble in pyridine and alcoholic potassium hydroxide giving each a deep red color. The residue was insoluble in other organic solvents. The residue which was insoluble in pyridine contained a high percentage of magnesium. The residue and alcohol were again placed in the silver bomb tube and heated four hours longer at 145 to 150°. There was no reaction.

A solution of 0.085 grams of rhodoporphyrin in 15 cc. of pyridine was prepared. To this solution 50 cc. of absolute ethyl alcohol and 0.060 grams of anhydrous magnesium acetate were added. The solution was placed in a silver bomb tube and heated five hours at 145 to 150°. The contents of the tube were filtered into 300 cc. of ether and washed five times with a dilute solution of mono-sodium phosphate. This was done to decompose any magnesium salt of rhodoporphyrin that might have formed during the above treatment. The ether solution was then washed many times with distilled water to remove all of the alcohol and mono-sodium phosphate. The ether solution was then filtered and evaporated to dryness. The residue was transferred to a previously weighed crucible, and dried at 105° and a pressure of 25 mm. of mercury. The residue was burned and the percentage of ash determined.

Anal. Subs., 0.0596: Ash, 0.0034. Calcd. for rhodophyllin: Ash, 0.0. Found 5.7.

The ash was dissolved in dilute hydrochloric acid, and the magnesium precipitated as the 8-hydroxyquinoline salt in the usual manner.

Anal. Subs., 0.0034. $\text{Mg}(\text{C}_9\text{H}_6\text{ON})_2$, 0.0226. Calcd. for MgO : Mg, 0.0020. Found 0.00175.

The percentage of ash for rhodophyllin is calculated on the basis that it contains 14% of ether of crystallization. This ether was not lost under the conditions used for drying the residue.¹

An Attempt to Remove Magnesium from Chlorophyll with 8-hydroxyquinoline.

To a solution of 0.2128 grams of chlorophyll dissolved in absolute alcohol, 2 cc. of a concentrated solution of ammonium hydroxide, and 25 cc. of 2% alcoholic 8-hydroxyquinoline were added. The solution was permitted to stand at room temperature for one month. No precipitate formed. The alcoholic solution was transferred to ether and washed thoroughly with distilled water to remove the alcohol and ammonia. The ether solution was then filtered and evaporated to 10 cc. To this solution was then added a large quantity of low boiling point ligroin. The precipitate was collected

1. Willstatter and Pfannenstiel, Ann., 358, 205 (1907)

and washed with more ligroin. This procedure removed the 8-hydroxyquinoline. The precipitate was transferred to a crucible and dried to constant weight in vacuo over phosphorus pentoxide, and then analyzed for ash.

Anal. Subs., 0.1464: Ash, 0.0065. Calcd. for chlorophyll: Ash, 4.5. Found 4.3.

An Attempt to Remove Magnesium from Synthetic Chlorophyll with 8-hydroxyquinoline.

The unknown chlorophyll derivative prepared by treating ethyl pheophorbide with magnesium acetate in alcoholic solution would not give up its magnesium when treated with 8 hydroxyquinoline in alkaline solution.

A solution of the unknown chlorophyll derivative containing 0.3856 grams in 75 cc. of ethyl alcohol was treated with ten cc. of 2% alcoholic 8-hydroxyquinoline and one cc. of concentrated ammonium hydroxide. The solution was permitted to stand at room temperature for a month. No precipitate had formed during this time. The unknown chlorophyll derivative was recovered and analyzed for ash. The 8-hydroxyquinoline was removed by the precipitation of the derivative from its concentrated ether solution with low boiling point ligroin.

Anal. Subs., 0.1574: Ash, 0.0078. Calcd. for ethyl chlorophyllide: Ash, 6.2. Found 4.9.

The analysis was low for ash when compared with the ash content of pure ethyl chlorophyllide, but the percentage of ash was as high as in previous analyses where no attempt was made to remove the magnesium.

Removal of Magnesium from Potassium Chlorophyllide with 8-hydroxyquinoline.

Potassium chlorophyllide was prepared with a high degree of purity by the saponification of pure chlorophyll with potassium hydroxide in absolute alcohol.

To a solution of 1.5 grams of pure chlorophyll dissolved in 200 cc. of absolute alcohol, 15 cc. of concentrated methyl alcoholic potassium hydroxide was added slowly with stirring. The solution was permitted to stand twelve hours. During this time the potassium chlorophyllide precipitated, and was collected on a filter and washed thoroughly with absolute alcohol to remove any free alkali. The yield was 0.4 grams.

The magnesium content of the pure potassium chlorophyllide was determined.

To a 0.1186 gram sample of pure potassium chlorophyllide in a large porcelain crucible, a few drops of concentrated sulfuric acid were added. The crucible was heated gradually to a temperature of 700°, and held there until all the carbon was burned. The ash was then dissolved in dilute hydrochloric acid, and the magnesium precipitated

as the magnesium salt of 8-hydroxyquinoline. The salt was transferred to a weighed crucible and washed with hot dilute ammonium hydroxide. The precipitate was then dried to constant weight at 130 to 140^o.

Anal. Subs., 0.1186: $\text{Mg}(\text{C}_9\text{H}_6\text{ON})_2$, 0.0412. Calcd. for potassium chlorophyllide: Mg, 3.0. Found 2.7.

A sample of the potassium chlorophyllide prepared above was treated with 8-hydroxyquinoline in alkaline solution, and it was found that the magnesium precipitated quantitatively as the magnesium salt of 8-hydroxyquinoline.

To a solution of 0.1350 grams of potassium chlorophyllide, prepared directly above, in 125 cc. of distilled water, 15 cc. of two percent alcoholic 8-hydroxyquinoline, and one cc. of concentrated ammonium hydroxide were added. A precipitate formed immediately. The solution was kept at room temperature for two days. The precipitate which was collected on a gooch filter was green in color. The precipitate was dissolved in acid and again precipitated with 8-hydroxyquinoline. The precipitate was transferred to a gooch crucible, dried at 130 to 140^o, and its weight determined.

Anal. Subs., 0.1350: $\text{Mg}(\text{C}_9\text{H}_6\text{ON})_2$, 0.0488. Calcd. for potassium chlorophyllide: Mg, 3.0. Found 2.8.

The purity of the magnesium salt of 8-hydroxyquinoline was confirmed by burning the salt with anhydrous oxalic acid which produces magnesium oxide.

Anal. Subs., 0.0488: MgO, 0.0062. Calcd. for
 $\text{Mg}(\text{C}_9\text{H}_6\text{ON})_2$: Mg, 7.8. Found 7.7.

An Attempt to Remove the Magnesium from Rhodophyllin
with 8-hydroxyquinoline.

To 0.1063 grams of rhodophyllin¹ suspended in 100 cc.
of distilled water, two cc. of concentrated ammonium
hydroxide were added. The mixture was shaken until all the
rhodophyllin had gone into solution. To this solution ten
cc. of a two percent alcoholic solution of 8-hydroxyquinoline
was added. The solution was kept at room temperature for
three days. No precipitate formed. The solution was heated.
A black precipitate formed from which no magnesium salt of
8-hydroxyquinoline could be obtained. The precipitate was
discarded.

An Attempt to Remove Magnesium from Pyrrophyllin with
8-hydroxyquinoline.

A 0.0786 gram sample of pyrrophyllin¹ was dissolved in
100 cc. of 90% ethyl alcohol. To this solution was added
0.5 grams of potassium hydroxide, and ten cc. of a two percent
alcoholic solution of 8-hydroxyquinoline. The solution was
permitted to stand six days. No precipitate formed. Heating
the solution also produced no precipitate.

1. Willstatter, "Investigations on Chlorophyll", transl.
by Schertz & Merz, 315 (1928).

SUMMARY

A study of the mode of attachment of magnesium in chlorophyll led to the following results:

1. Magnesium was introduced into pheophytin with magnesium acetate in alcoholic solution.
2. Magnesium was introduced into ethyl pheophorbides (a) and (b) with magnesium acetate in alcoholic solution.
3. Magnesium was introduced into rhodoporphyrin with magnesium acetate in alcoholic solution.
4. Magnesium was introduced into pyrroporphyrin with magnesium acetate in alcoholic solution.
5. Glaucophyllin was partially synthesized.
6. The magnesium could not be removed from chlorophyll with 8-hydroxyquinoline in alkaline solution.
7. The magnesium was removed quantitatively from potassium chlorophyllide with 8-hydroxyquinoline in alkaline solution.
8. The magnesium could not be removed from rhodophyllin with 8-hydroxyquinoline in alkaline solution.
9. The magnesium could not be removed from pyrrophyllin with 8-hydroxyquinoline in alkaline solution.

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